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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 31/445, C07D 401/06, 401/14, 405/06, 405/10

A1

(11) International Publication Number: **WO 96/32110**

(43) International Publication Date: 17 October 1996 (17.10.96)

(21) International Application Number: PCT/US96/04679

(22) International Filing Date: 4 April 1996 (04.04.96)

(30) Priority Data:
08/419,683 10 April 1995 (10.04.95) US

(60) Parent Application or Grant
(63) Related by Continuation
US 08/419,683 (CIP)
Filed on 10 April 1995 (10.04.95)

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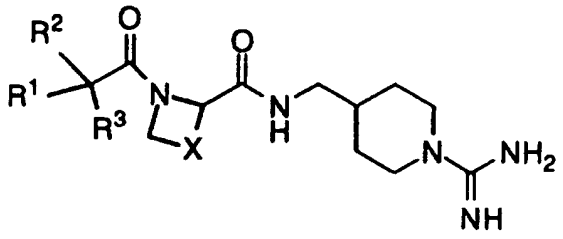
(81) Designated States: AL, AM, AU, AZ, BB, BG, BR, BY, CA, CN, CZ, EE, GE, HU, IS, JP, KG, KR, KZ, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

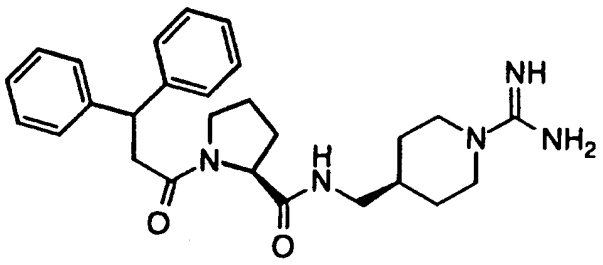
(54) Title: THROMBIN INHIBITORS

(57) Abstract

Coumpounds of the invention have structure (I), for example (II). These compounds inhibit thrombin and associated thrombosis.



(I)



(II)

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TITLE OF THE INVENTION
THROMBIN INHIBITORS

BACKGROUND OF THE INVENTION

Thrombin is a serine protease present in blood plasma in the form of a precursor, prothrombin. Thrombin plays a central role in the mechanism of blood coagulation by converting the solution plasma protein, fibrinogen, into insoluble fibrin.

Edwards et al. *J. Amer. Chem. Soc.* (1992) vol. 114, pp. 1854-63, describes peptidyl a-ketobenzoxazoles which are reversible inhibitors of the serine proteases human leukocyte elastase and porcine pancreatic elastase.

European Publication 363 284 describes analogs of peptidase substrates in which the nitrogen atom of the scissile amide group of the substrate peptide has been replaced by hydrogen or a substituted carbonyl moiety.

Australian Publication 86245677 also describes peptidase inhibitors having an activated electrophilic ketone moiety such as fluoromethylene ketone or a-keto carboxyl derivatives.

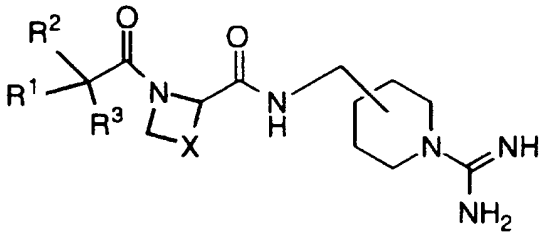
Thrombin inhibitors described in prior publications contain sidechains of arginine and lysine. These structures show low selectivity for thrombin over other trypsin-like enzymes. Some of them show toxicity of hypotension and liver toxicity.

European Publication 601 459 describes sulfonamido heterocyclic thrombin inhibitors, such as N-[4-[(aminoiminomethyl)amino]butyl]-1-[N-(2-naphthalenylsulfonyl)-L-phenylalanyl]-L-prolinamide.

WO 94/29336 describes compounds which are useful as thrombin inhibitors.

SUMMARY OF THE INVENTION

Compounds of the invention have the following structure:



wherein

R^1 and R^2 are independently

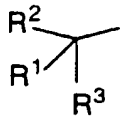
hydrogen,
phenyl unsubstituted, mono- or di-substituted with OH, C_{1-4} alkyl, oxo, or halo,
naphthyl,
biphenyl,
a 5- to 7- membered mono- or bicyclic heterocyclic ring or bicyclic heterocyclic ring system any ring of which may be saturated or unsaturated, and which consists of carbon atoms and from one to three heteroatoms selected from the group consisting of N, O and S,
C_{1-4} alkyl,
branched C_{1-4} alkyl,
C_{3-7} cycloalkyl,
C_{5-12} bicyclic alkyl,
C_{11-16} tricyclic alkyl,
$R^4(CH_2)_n$,
$(R^4)_2CH$, wherein R^4 is the same or different,
$(R^4)(R^4O)CH$, wherein R^4 is the same or different,
$R^4O(CH_2)_n$, or
R^2 may be joined with R^1 to form a four- to seven membered carbon ring in which zero to two carbon atoms may be substituted with heteroatoms independently selected from the list N, O, and S,

where n is 1, 2, 3 or 4; and

R^3 is

hydrogen, or
$HO(CH_2)_p$, where p is 0, 1, 2, 3 or 4;

or



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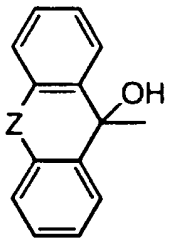


wherein Z is C, O or a bond;

R^4 is

phenyl unsubstituted, mono- or di-substituted with OH, OMe, C_{1-4} alkyl, oxo, or halo,
naphthyl,
biphenyl,
a 5- to 7- membered mono- or bicyclic heterocyclic ring or bicyclic heterocyclic ring system any ring of which may be saturated or unsaturated, and which consists of carbon atoms and from one to three heteroatoms selected from the group consisting of N, O and S,
C_{1-4} alkyl,
branched C_{1-4} alkyl,
C_{3-7} cycloalkyl,
C_{5-12} bicyclic alkyl, or
C_{11-16} tricyclic alkyl; and

X is $(CH_2)_q$ where q is 1 or 2,
NR^1CH_2, or

SCH_2,

and pharmaceutically acceptable salts thereof.

These compounds show selectivity for thrombin inhibition over inhibition of trypsin and other trypsin-like enzymes and have oral bioavailability. Trypsin-like enzymes (such as trypsin, thrombin, factor xa, kallikrein, plasmin, urokinase, and plasminogen activator) are serine dependent enzymes that catalyze hydrolysis at arginyl and lysyl peptide bonds.

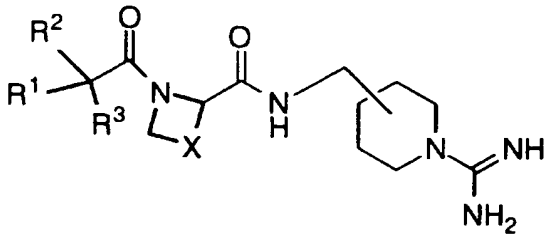
The invention includes a composition for inhibiting loss of blood platelets, inhibiting formation of blood platelet aggregates, inhibiting formation of fibrin, inhibiting thrombus formation, and inhibiting embolus formation in a mammal, comprising a compound of the invention in a pharmaceutically acceptable carrier. These compositions may optionally include anticoagulants, antiplatelet agents, and thrombolytic agents. The compositions can be added to blood, blood products, or mammalian organs in order to effect the desired inhibitions.

The invention also includes a composition for preventing or treating unstable angina, refractory angina, myocardial infarction, transient ischemic attacks, atrial fibrillation, thrombotic stroke, embolic stroke, deep vein thrombosis, disseminated intravascular coagulation, ocular build up of fibrin, and reocclusion or restenosis of recanalized vessels, in a mammal, comprising a compound of the invention in a pharmaceutically acceptable carrier. These compositions may optionally include anticoagulants, antiplatelet agents, and thrombolytic agents.

The invention also includes a method for reducing the thrombogenicity of a surface in a mammal by attaching to the surface, either covalently or noncovalently, a compound of the invention.

DETAILED DESCRIPTION OF THE INVENTION

Compounds of the invention have the following structure:



wherein

R^1 and R^2 are independently

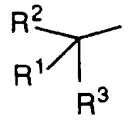
hydrogen,
phenyl unsubstituted, mono- or di-substituted with OH, C_{1-4} alkyl, oxo, or halo,
naphthyl,
biphenyl,
a 5- to 7- membered mono- or bicyclic heterocyclic ring or bicyclic heterocyclic ring system any ring of which may be saturated or unsaturated, and which consists of carbon atoms and from one to three heteroatoms selected from the group consisting of N, O and S,
C_{1-4} alkyl,
branched C_{1-4} alkyl,
C_{3-7} cycloalkyl,
C_{5-12} bicyclic alkyl,
C_{11-16} tricyclic alkyl,
$R^4(CH_2)_n$,
$(R^4)_2CH$, wherein R^4 is the same or different,
$(R^4)(R^4O)CH$, wherein R^4 is the same or different,
$R^4O(CH_2)_n$, or
R^2 may be joined with R^1 to form a four- to seven membered carbon ring in which zero to two carbon atoms may be substituted with heteroatoms independently selected from the list N, O, and S,

where n is 1, 2, 3 or 4; and

R^3 is

hydrogen, or
$HO(CH_2)_p$, where p is 0, 1, 2, 3 or 4;

or



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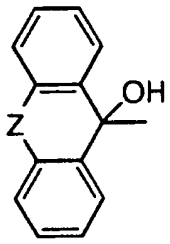


wherein Z is C, O or a bond;

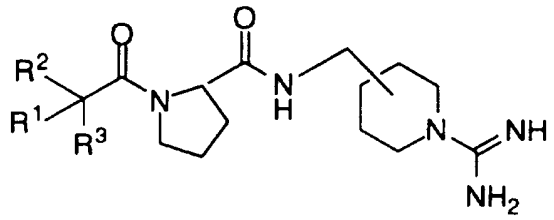
R^4 is

phenyl unsubstituted, mono- or di-substituted with OH, OMe, C_{1-4} alkyl, oxo, or halo,
naphthyl,
biphenyl,
1-dibenzocycloheptene,
a 5- to 7- membered mono- or bicyclic heterocyclic ring or bicyclic heterocyclic ring system any ring of which may be saturated or unsaturated, and which consists of carbon atoms and from one to three heteroatoms selected from the group consisting of N, O and S,
C_{1-4} alkyl,
branched C_{1-4} alkyl,
C_{3-7} cycloalkyl,
C_{5-12} bicyclic alkyl, or
C_{11-16} tricyclic alkyl; and

X is $(CH_2)_q$ where q is 1 or 2,
NR^1CH_2, or
SCH_2,

and pharmaceutically acceptable salts thereof.

In one class of compounds of the invention, the compounds have the structure



wherein

R^1 and R^2 are independently

hydrogen,

phenyl unsubstituted, mono- or di-substituted with OH, C_{1-4} alkyl, oxo, or halo,

naphthyl,

biphenyl,

a 5- to 7- membered mono- or bicyclic heterocyclic ring or bicyclic heterocyclic ring system any ring of which may be saturated or unsaturated, and which consists of carbon atoms and from one to three heteroatoms selected from the group consisting of N, O and S,

C_{1-4} alkyl,

branched C_{1-4} alkyl,

C_{3-7} cycloalkyl,

C_{5-12} bicyclic alkyl,

C_{11-16} tricyclic alkyl,

$R^4(CH_2)_n$,

$(R^4)_2CH$, wherein R^4 is the same or different,

$(R^4)(R^4O)CH$, wherein R^4 is the same or different,

$R^4O(CH_2)_n$, or

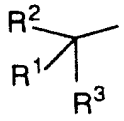
R^2 may be joined with R^1 to form a four- to seven membered carbon ring in which zero to two carbon atoms may be substituted with heteroatoms independently selected from the list N, O, and S.

where n is 1, 2, 3 or 4; and

R^3 is

hydrogen, or
$HO(CH_2)_p$, where p is 0, 1, 2, 3 or 4;

or



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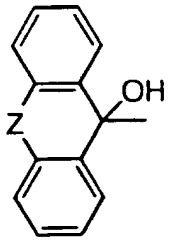


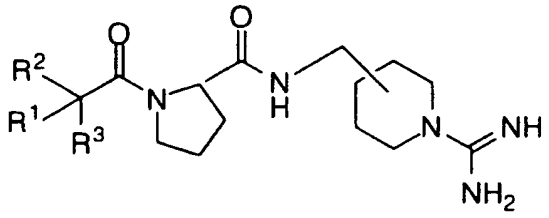
wherein Z is C, O or a bond;

R^4 is

phenyl unsubstituted, mono- or di-substituted with OH, OMe, C_{1-4} alkyl, oxo, or halo,
naphthyl,
biphenyl,
1-dibenzocycloheptene,
a 5- to 7- membered mono- or bicyclic heterocyclic ring or bicyclic heterocyclic ring system any ring of which may be saturated or unsaturated, and which consists of carbon atoms and from one to three heteroatoms selected from the group consisting of N, O and S,
C_{1-4} alkyl,
branched C_{1-4} alkyl,
C_{3-7} cycloalkyl,
C_{5-12} bicyclic alkyl, or
C_{11-16} tricyclic alkyl.

and pharmaceutically acceptable salts thereof.

In a subclass of this class of compounds of the invention, the compounds have the structure



wherein

R^1 and R^2 are independently

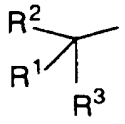
hydrogen,
phenyl unsubstituted, mono- or di-substituted with OH,
C_{1-4} alkyl, oxo, or halo,
$(R^4)_2CH$, wherein R^4 is the same or different,
R^2 may be joined with R^1 to form a four- to seven membered carbon ring in which zero to two carbon atoms may be substituted with heteroatoms independently selected from the list N, O, and S.

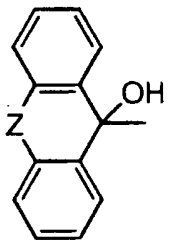
where n is 1, 2, 3 or 4; and

R^3 is

hydrogen, or
$HO(CH_2)_p$, where p is 0, 1, 2, 3 or 4;

or



is 

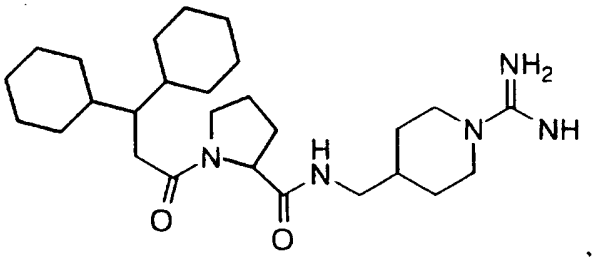
wherein Z is C, O or a bond;

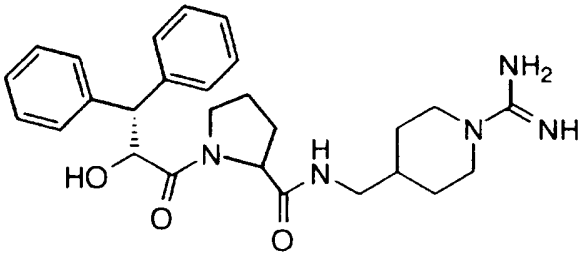
R^4 is

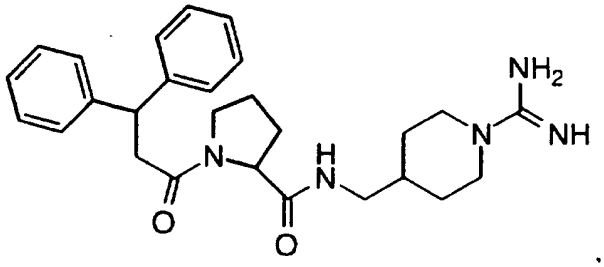
phenyl unsubstituted, mono- or di-substituted with OH, OMe, C_{1-4} alkyl, oxo, or halo,
1-dibenzocycloheptene,
a 6- membered monocyclic heterocyclic ring which may be saturated or unsaturated, and which consists of carbon atoms and from one to three N heteroatoms, or
C_{3-7} cycloalkyl,

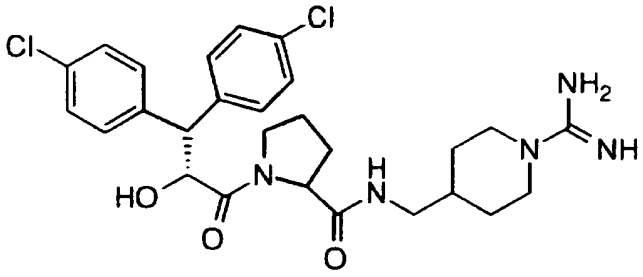
and pharmaceutically acceptable salts thereof.

A group of compounds of this subclass include









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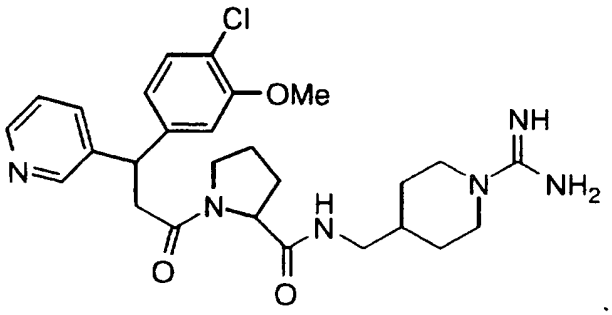

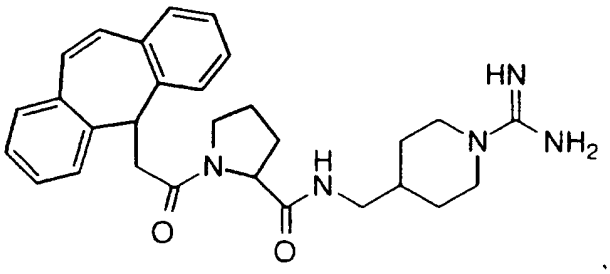

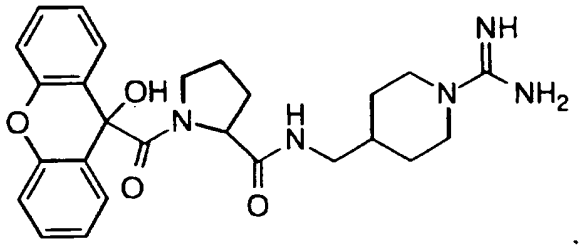

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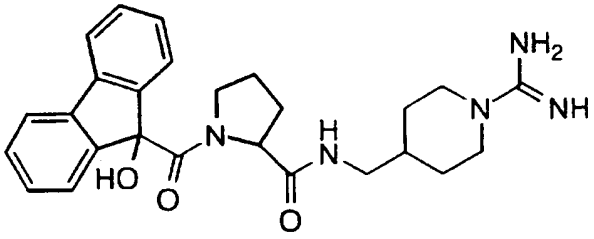
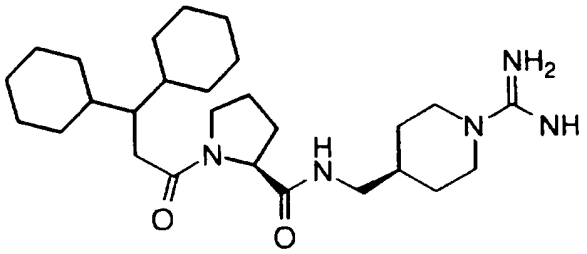


,

and pharmaceutically acceptable salts thereof.

Specific embodiments of this group include



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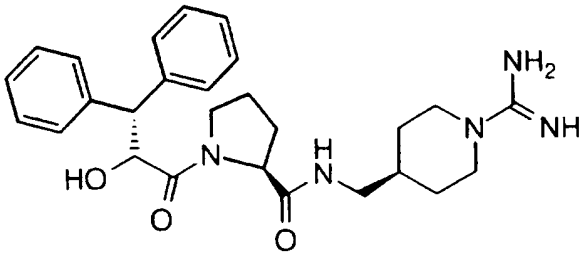

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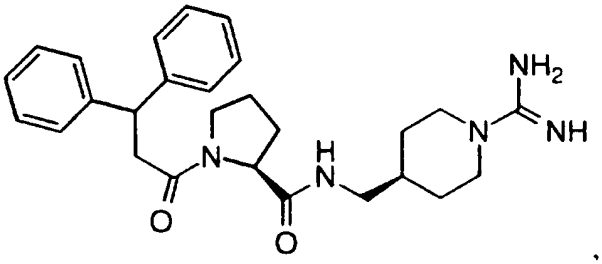

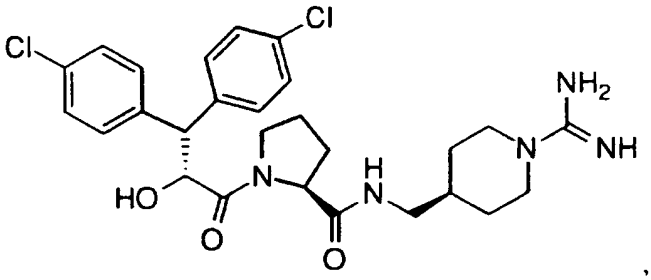

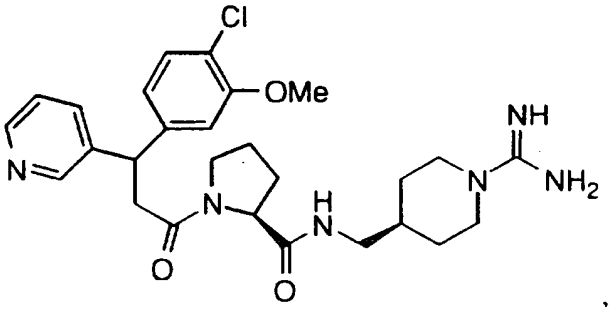

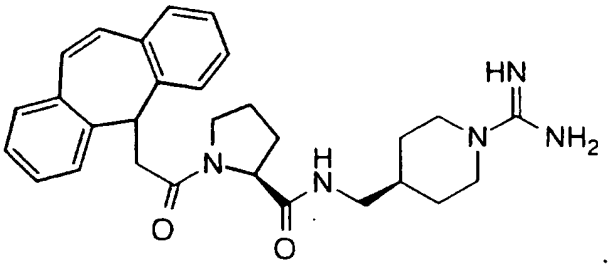

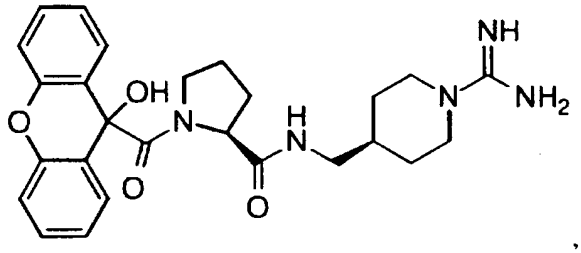

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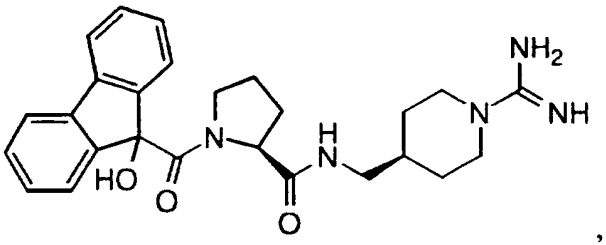

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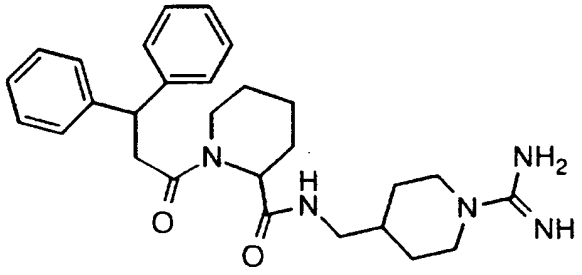
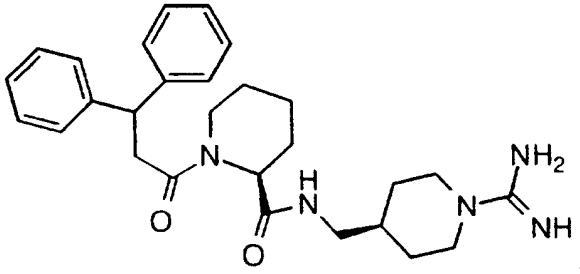
and pharmaceutically acceptable salts thereof.

Another compound of the invention is



and pharmaceutically acceptable salts thereof.

An embodiment of the compound is



and pharmaceutically acceptable salts thereof.

Some abbreviations that may appear in this application are as follows.

ABBREVIATIONS

Designation	Protecting Group
BOC (Boc)	t-butyloxycarbonyl
CBZ (Cbz)	benzyloxycarbonyl(carbobenzoxy)
TBS (TBDMS)	t-butyl-dimethylsilyl
Activating Group	
HBT(HOBT or HOBt)	1-hydroxybenzotriazole hydrate
Designation	**Coupling Reagent**
BOP reagent	benzotriazol-1-yloxytris-(dimethylamino)phosphonium hexafluorophosphate
BOP-Cl	bis(2-oxo-3-oxazolidinyl)phosphinic chloride
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
	Other
$(BOC)_2O$ (BOC_2O)	di-t-butyl dicarbonate
n-Bu_4N+F-	tetrabutyl ammonium fluoride
nBuLi (n-Buli)	n-butyllithium
DMF	dimethylformamide
Et_3N	triethylamine
EtOAc	ethyl acetate
TFA	trifluoroacetic acid
DMAP	dimethylaminopyridine
DME	dimethoxyethane
LDA	lithium diisopropylamide
THF	tetrahydrofuran

	Amino Acid
Ile	Isoleucine
Phe	Phenylalanine
Pro	Proline
Ala	Alanine
Val	Valine

The compounds of the present invention may have chiral centers and occur as racemates, racemic mixtures and as individual diastereomers, or enantiomers with all isomeric forms being included in the present invention.

When any variable occurs more than one time in any constituent or in formula I, its definition on each occurrence is independent of its definition at every other occurrence. Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

As used herein except where noted, "alkyl" is intended to include both branched- and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms (Me is methyl, Et is ethyl, Pr is propyl, Bu is butyl); "alkoxy" represents an alkyl group of indicated number of carbon atoms attached through an oxygen bridge; "Halo", as used herein, means fluoro, chloro, bromo and iodo; and "counterion" is used to represent a small, single negatively-charged species, such as chloride, bromide, hydroxide, acetate, trifluroacetate, perchlorate, nitrate, benzoate, maleate, tartrate, hemitartrate, benzene sulfonate, and the like.

The term heterocycle or heterocyclic, as used herein except where noted, represents a stable 5- to 7-membered mono- or bicyclic or stable 7- to 10-membered bicyclic heterocyclic ring system any ring of which may be saturated or unsaturated, and which consists of carbon atoms and from one to three heteroatoms selected from the group consisting of N, O and S, and wherein the nitrogen and sulfur heteroatoms may optionally be oxidized, and the nitrogen heteroatom

may optionally be quaternized, and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. The heterocyclic ring may be attached at any heteroatom or carbon atom which results in the creation of a stable structure. Examples of such heterocyclic elements include piperidinyl, piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolodinyl, 2-oxoazepinyl, azepinyl, pyrrolyl, 4-piperidonyl, pyrrolidinyl, pyrazolyl, pyrazolidinyl, imidazolyl, imidazolinyl, imidazolidinyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, oxazolyl, oxazolidinyl, isoxazolyl, isoxazolidinyl, morpholinyl, thiazolyl, thiazolidinyl, isothiazolyl, quinuclidinyl, isothiazolidinyl, indolyl, quinolinyl, isoquinolinyl, benzimidazolyl, thiadiazoyl, benzopyranyl, benzothiazolyl, benzoxazolyl, furyl, tetrahydrofuryl, tetrahydropyranyl, thienyl, benzothienyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiamorpholinyl sulfone, and oxadiazolyl. Morpholino is the same as morpholinyl.

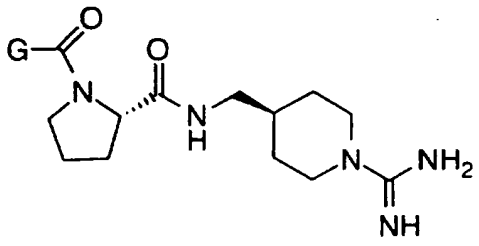
The pharmaceutically-acceptable salts of the compounds of Formula I (in the form of water- or oil-soluble or dispersible products) include the conventional non-toxic salts or the quaternary ammonium salts which are formed, e.g., from inorganic or organic acids or bases. Examples of such acid addition salts include acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, and undecanoate. Base salts include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth. Also, the basic nitrogen-containing groups may be quaternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and

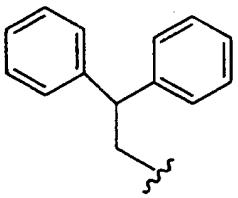
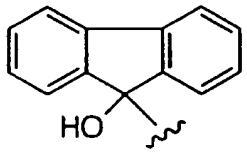
butyl chloride, bromides and iodides; dialkyl sulfates like dimethyl, diethyl, dibutyl; and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides like benzyl and phenethyl bromides and others.

Amide couplings used to form the compounds of this invention are typically performed by the carbodiimide method with reagents such as dicyclohexylcarbodiimide, or 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide. Other methods of forming the amide or peptide bond include, but are not limited to the synthetic routes via an acid chloride, azide, mixed anhydride or activated ester. Typically, solution phase amide coupling are performed, but solid-phase synthesis by classical Merrifield techniques may be employed instead. The addition and removal of one or more protecting groups is also typical practice.

The compounds shown in the tables below are exemplary compounds of the present invention:

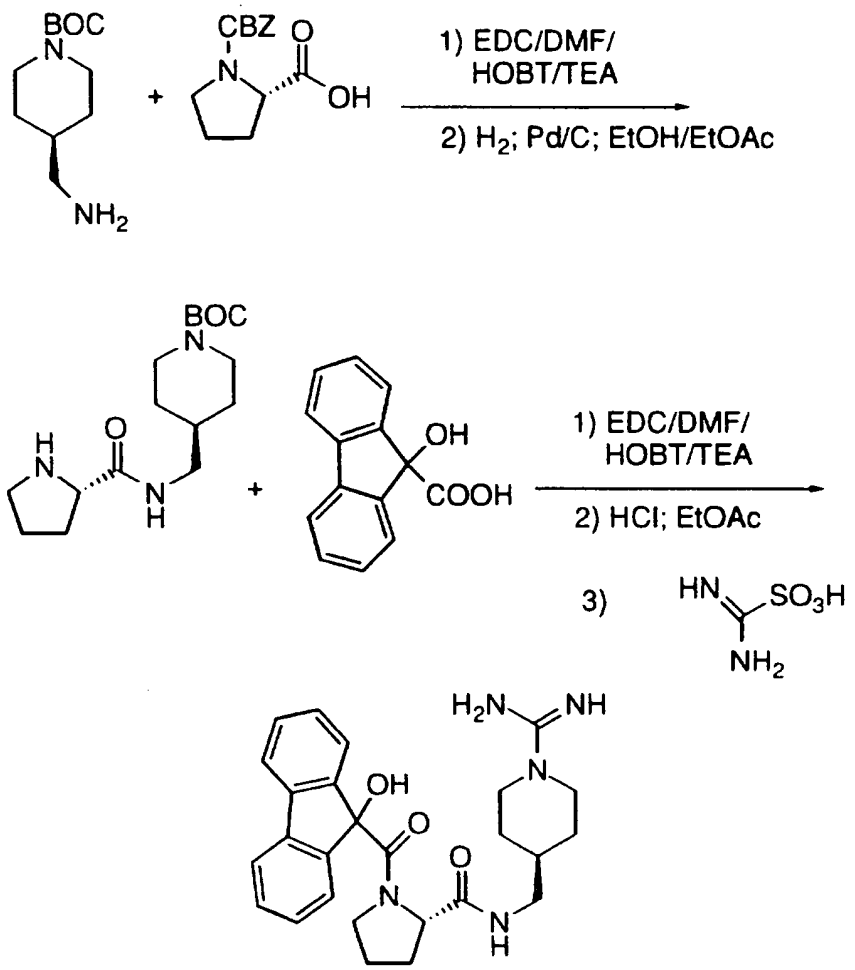
TABLE 2



G	Ki (nM vs thrombin)
	2.7
	1.1

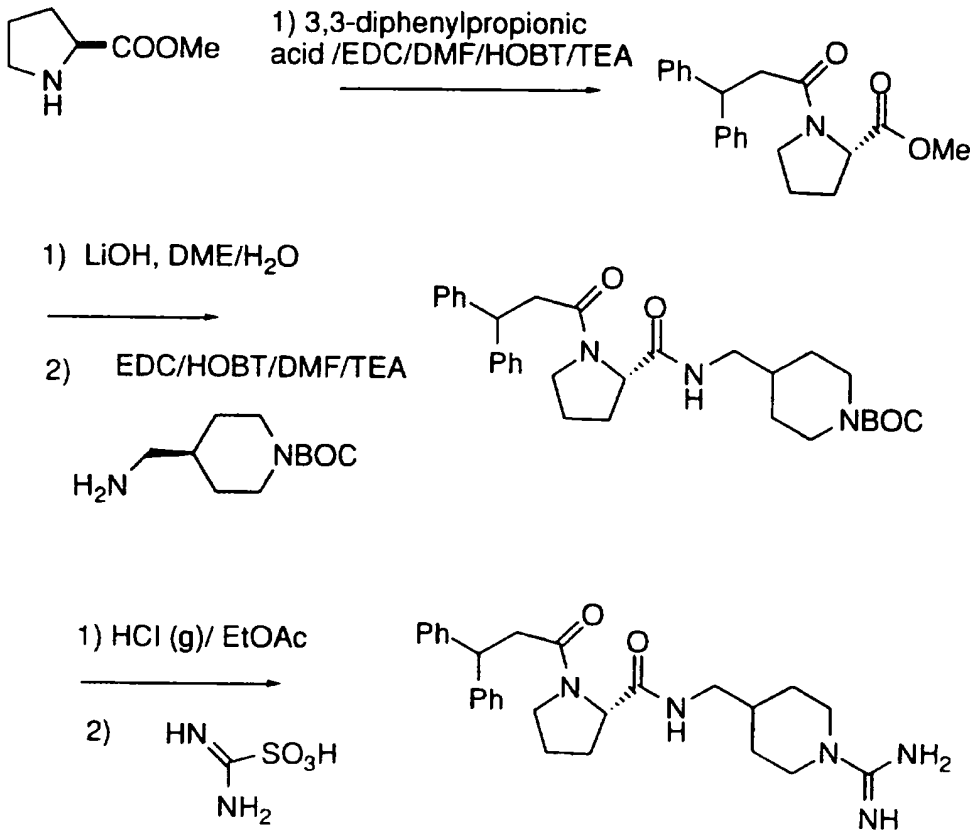
The following synthetic routes can be used to prepare compounds of the invention. Using method I 4-aminomethyl-BOC-piperidine is coupled to CBZ-L-proline using standard amide coupling procedures. The CBZ group is then removed via hydrogenation and the proline amine is coupled with an acid such as 9-hydroxy-flourene-carboxylic acid. The BOC group is removed from the piperidine, and the amine is converted to the guanidine using a guanylating reagent such as amidinosulfonic acid.

METHOD 1



A second method for constructing the compounds of general structure I (as exemplified by examples 1 and 2) is to react an acid such as 9-hydroxy-flourene-carboxylic acid or 3,3-diphenyl-propionic acid with L-proline-methyl ester. The ester group is hydrolyzed and the acid is then coupled with 4-aminomethyl-Boc-Piperidine. The BOC group is removed with an acid such as HCl or trifluoroacetic acid, and the resultant amine is reacted with amidine-sulfonic acid to afford the desired product.

METHOD 2


COOMe
N
H
1) 3,3-diphenylpropionic acid /EDC/DMF/HOBT/TEA
Ph
Ph
O
O
N
OMe
1) LiOH, DME/H2O
2) EDC/HOBT/DMF/TEA
H2N
NBOC
Ph
Ph
O
O
N
N
H
NBOC
1) HCl (g)/ EtOAc
2)
HN
SO3H
NH2
Ph
Ph
O
O
N
N
H
N
NH2
NH

EXAMPLE 1

Preparation of N'-[[1-(Aminoiminomethyl)-4-piperidinyl]methyl]-N-(3,3-diphenylpropionyl)-L-proline amide

Step A: N-(3,3-diphenylpropionyl-L-proline methylester

To a solution of 3,3 Diphenylpropionic acid (2.00 g, 8.85 mmol) in DMF (20 mL) was added EDC (2.04 g, 10.62 mmol), HOBT (1.44 g, 10.62 mmol) and L-proline methyl ester hydrochloride (1.76 g, 10.62 mmol) with stirring at ambient temperature. Triethylamine (2.96 mL, 21.24 mmol) was added to adjust the pH of the mixture to 8.5. After 24 hrs the DMF was removed under reduced pressure and the residue was partitioned between EtOAc (100 mL)- H_2O (50 mL). The aqueous layer was washed with EtOAc (2x 50 mL), organics combined, washed with 10% citric acid soln, aq satd $NaHCO_3$ soln, brine and dried (Na_2SO_4). Filtration and concentration to dryness gave 3.50 g of crude product which was chromatographed (SiO_2) eluting with 99 CH_2Cl_2:1 MeOH to 98 CH_2Cl_2:2 MeOH to give 2.48 g (83%) of N-(3,3-diphenylpropionyl)-L-proline methylester.

Step B: N-(3,3-diphenylpropionyl)-L-proline

N-(3,3-diphenylpropionyl)-L-proline methylester (2.10 g, 6.23 mmol) was dissolved in DME (50 ml) and H_2O (50 ml). Lithium hydroxide monohydrate (1.57 g, 37.39 mmol) was added and the mixture was stirred at ambient temperature for 2 hrs. The reaction mixture was concentrated to remove DME and the remaining aqueous layer was acidified with 1N HCl to pH 3. The acidic layer was washed with EtOAc (3X75 ml). Organic washes were combined, dried with Na_2SO_4, filtered and concentrated to give 1.92 g (94%) of N-(3,3-diphenylpropionyl)-L-proline.

Step C: N'-[[1-(t-Butyloxycarbonyl)-4-piperidinyl]methyl]-N-(3,3-diphenylpropionyl-L-proline amide

To a solution of N-(3,3-diphenylpropionyl)-L-proline (0.600 g, 1.83 mmol) in DMF (8 mL) was added EDC (0.422 g, 2.20 mmol), HOBT (0.297 g, 2.20 mmol) and N-1-(t-butyloxycarbonyl) aminomethyl piperidine (0.471 g, 2.20 mmol) with stirring at ambient temperature. Triethylamine (0.307 mL, 2.20 mmol) was added to adjust the pH of the mixture to 8.5. After 24 hrs the DMF was removed under reduced pressure and the residue was partitioned between EtOAc (50 mL)- H_2O (25 mL). The aqueous layer was washed with EtOAc (2x 25 mL), organics combined, washed with aq satd $NaHCO_3$ soln, brine and dried (Na_2SO_4). Filtration and concentration to dryness gave .960 g of crude product which was chromatographed (SiO_2) eluting with 99 CH_2Cl_2:1 MeOH to 98 CH_2Cl_2:2 MeOH to give .750 g (79%) of N'-[[1-(t-Butyloxycarbonyl)-4-piperidinyl]methyl]-N-(3,3-diphenylpropionyl)-L-proline amide.

Step D: N'-[4-piperidinylmethyl]-N-(3,3-diphenylpropionyl)-L-proline amide hydrochloride

HCl gas was bubbled into a soln of N'-[[1-(t-Butyloxy-carbonyl)-4-piperidinyl]methyl]-N-(3,3-diphenylpropionyl)-L-proline amide (0.750 g, 1.45 mmol) in EtOAc (75 mL) at -20°C for 5 min. The mixture was capped and allowed to stir at ambient temperature for 1 hr. The solution was concentrated to dryness to give 0.656 g (100%) of N'-[4-piperidinylmethyl]-N-(3,3-diphenylpropionyl)-L-proline amide hydrochloride which was used without further purification.

Step E: N'-[[1-(Aminoiminomethyl)-4-piperidinyl]methyl]-N-(3,3-diphenylpropionyl)-L-proline amide

N'-[4-piperidinylmethyl]-N-(3,3-diphenylpropionyl)-L-proline amide hydrochloride (0.650 g, 0.1.43 mmol) was dissolved in DMF (6 mL) and treated with triethylamine (0.438 ml, 3.15 mmol) and amidine sulfonic acid (0.195 g, 1.57 mmol) with stirring at ambient temperature. After stirring for 72 hrs and removal of the DMF, the

residue was purified by prep. HPLC. Pure fractions were combined and lyopholized to give 440 mg of N'-[[1-(Aminoiminomethyl)-4-piperidinyl]methyl]-N-(3,3-diphenylpropionyl)-L-proline amide.

EXAMPLE 2

Preparation of N'-[[1-(Aminoiminomethyl)-4-piperidinyl]methyl]-N-(9-hydroxyfluorene-9-carboxyl)-L-proline amide

Step A: N-(9-hydroxyfluorene-9-carboxyl)-L-proline methylester

To a solution of L-proline methyl ester hydrochloride (0.225 g, 1.36 mmol) in dimethylacetamide (DMA, 15 mL) was added EDC (0.366 g, 1.91 mmol), HOBT (0.251 g, 1.64 mmol) and 9-hydroxy-fluorene-9-carboxylic acid (0.371 g, 1.64 mmol) with stirring at ambient temperature. N-methylmorpholine was added to adjust the pH of the mixture to 7.5-8. After 5 days the DMA was removed under reduced pressure and the residue was partitioned between EtOAc (100 mL)- H_2O (50 mL). The aqueous layer was washed with EtOAc (2x 50 mL), organics combined, washed with 10% $KHSO_4$ soln, 2X water, 1X aq satd $NaHCO_3$ soln, 2X brine and dried (Na_2SO_4). Filtration and concentration to dryness gave 0.538 g of crude product which was used as is in the next reaction.

Step B: N-(9-hydroxyfluorene-9-carboxyl)-L-proline

N-(9-hydroxyfluorene-9-carboxyl)-L-proline methylester (0.539 g, 1.6 mmol) was dissolved in 20 ml of 50% THF and H_2O. 1.0 ml (2 mmol) of a 2N Lithium hydroxide solution was added and the mixture was stirred at ambient temperature for 4.5 hrs. The reaction mixture was followed to completion via TLC (12:2:2:10; EtOAc:HOAc:IsoOct:H_2O). The mixture was then adjusted to pH<2 with $KHSO_4$ solution and partitioned between EtOAc (100 mL) and H_2O (50 mL). The aqueous layer was removed and the EtOAc washed 2X with brine, 1X H_2O, dried over Na_2SO_4, filtered and concentrated to afford 400 mg of (90%) of N-(9-hydroxyfluorene-9-carboxyl)-L-proline.

Step C: N'-[[1-(t-Butyloxycarbonyl)-4-piperidinyl]methyl]-N-(9-hydroxyfluorene-9-carboxyl)-L-proline amide

To a solution of N-1-(t-butyloxycarbonyl) aminomethyl piperidine (0.308 g, 1.44 mmol) in DMF (15 mL) was added EDC (0.395 g, 2.06 mmol), HOBT (0.265 g, 1.73 mmol) and N-(9-hydroxyfluorene-9-carboxyl)-L-proline (0.557 g, 1.72 mmol) with stirring at ambient temperature. N-methylmorpholine was added to adjust the pH of the mixture to 7.5-8. After 5 days an additional amount of N-(9-hydroxy-fluorene-9-carboxyl)-L-proline (1.15 mmol), HOBT (0.32 mmol) and EDC (1.44 mmol) was added to the reaction. After an additional 24 hrs, the DMF was removed under reduced pressure and the residue was partitioned between EtOAc (100 mL)- H_2O (75 mL). The aqueous layer was washed with EtOAc (2x 50 mL), organics combined, washed with 10% $KHSO_4$ solution, 2X H_2O, 1X 10% $NaHCO_3$ solution, 1X brine and dried over Na_2SO_4. Filtration and concentration to dryness gave 1.068 g of crude product which was 49% pure by reverse phase HPLC.

Step D: N'-[4-piperidinylmethyl]-N-(9-hydroxyfluorene-9-carboxyl)-L-proline amide trifluoroacetate

1.068 g of crude N'-[[1-(t-Butyloxycarbonyl)-4-piperi-dinyl]methyl]-N-(9-hydroxyfluorene-9-carboxyl)-L-proline amide was dissolved in 40 ml of 50% trifluoroacetic acid/50% methylene chloride and mixed at room temperature for 25 minutes. The TFA/CH_2Cl_2 was removed under reduced pressure and the residue yielded 1.07 g of crude product which was 40% pure via HPLC. This was used in the next step without further purification.

Step E: N'-[[1-(Aminoiminomethyl)-4-piperidinyl]methyl]-N-(9-hydroxyfluorene-9-carboxyl)-L-proline amide

N'-[4-piperidinylmethyl]-N-(9-hydroxyfluorene-9-carboxyl)-L-proline amide trifluoroacetate (1.07 g) was dissolved in DMF (20 mL) and treated with triethylamine (0.440 ml, 3.54 mmol) and amidine sulfonic acid (0.199 g, 1.61 mmol) with stirring at ambient temperature. After two days, an additional amount of amidine sulfonic acid (0.184 g,

1.48 mmol) and triethylamine (0.205 mL, 1.48 mmol) was added. After stirring for a total of four days, the DMF was removed and the residue was purified by prep. HPLC. Pure fractions were combined and lyopholized to give 114 mgs of N'-[[1-(Aminoiminomethyl)-4-piperidinyl]methyl]-N-(9-hydroxyfluorene-9-carboxyl)-L-proline amide trifluoroacetate.

In Vitro Assay For Determining Proteinase Inhibition

Assays of human a-thrombin and bovine trypsin were performed at 25°C in 0.05 M TRIS buffer pH 7.4, 0.15 M NaCl, 0.1% PEG. Trypsin assays also contained 1 mM $CaCl_2$. In assays wherein rates of hydrolysis of a *p*-nitroanilide (pna) substrate were determined, a Thermomax 96-well plate reader was used was used to measure (at 405 nm) the time dependent appearance of *p*-nitroaniline. sar-PR-pna was used to assay human a-thrombin (K_m=125 μM) and bovine trypsin (K_m=125 μM). *p*-Nitroanilide substrate concentration was determined from measurements of absorbance at 342 nm using an extinction coefficient of 8270 $cm^{-1}M^{-1}$.

In certain studies with potent inhibitors (K_i < 10 nM) where the degree of inhibition of thrombin was high, a more sensitive activity assay was employed. In this assay the rate of thrombin catalyzed hydrolysis of the fluorogenic substrate Z-GPR-afc (Cbz-Gly-Pro-Arg-7-amino-4-trifluoromethyl coumarin) (K_m=27 μM) was determined from the increase in fluorescence at 500 nm (excitation at 400 nm) associated with production of 7-amino-4-trifluoromethyl coumarin. Concentrations of stock solutions of Z-GPR-afc were determined from measurements of absorbance at 380 nm of the 7-amino-4-trifluoromethyl coumarin produced upon complete hydrolysis of an aliquot of the stock solution by thrombin.

Activity assays were performed by diluting a stock solution of substrate at least tenfold to a final concentration ≤ 0.1 K_m into a solution containing enzyme or enzyme equilibrated with inhibitor. Times required to achieve equilibration between enzyme and inhibitor were determined in control experiments. Initial velocities of product formation

in the absence (V_o) or presence of inhibitor (V_i) were measured. Assuming competitive inhibition, and that unity is negligible compared $K_m/[S]$, $[I]/e$, and $[I]/e$ (where [S], [I], and e respectively represent the total concentrations, of substrate, inhibitor and enzyme), the equilibrium constant (K_i) for dissociation of the inhibitor from the enzyme can be obtained from the dependence of V_o/V_i on [I] shown in equation 1.

$$V_o/V_i = 1 + [I]/K_i \qquad (1)$$

The activities shown by this assay indicate that the compounds of the invention are therapeutically useful for treating various conditions in patients suffering from unstable angina, refractory angina, myocardial infarction, transient ischemic attacks, atrial fibrillation, thrombotic stroke, embolic stroke, deep vein thrombosis, disseminated intravascular coagulation, and reocclusion or restenosis of recanalized vessels.

In Vivo Studies To Measure Thombotic Occlusions

Applicants have conducted *in vivo* studies of the compounds claimed herein using the following rat ferric chloride assay.

In the assay used to determine *in vivo* activity of the thrombin inhibitors or the invention, Male Sprague-Dawley rats (body weights 200-350 grams) were anesthetized with dial-urethane solution (0.1 ml/100 gm body weight i.p.), and a lateral tail vein was cannulated with a 23 gauge needle connected to a 12 inch length of PE50 tubing. The tubing was attached to a 3-way valve by a tubing adapter. Saline (control) or test compound, as appropriate, was administered via the tail vein catheter. A tracheostomy was performed with a 0.75 inch length of PE205 tubing. The right carotid artery was exposed and a 1.3 mm diameter Doppler flow probe was placed on the vessel. Body temperature was maintained at 37°C using a heat lamp.

Rats (8-10/group) were randomized to continuous intravenous infusions of saline or test compound administered via the tail vein at a rate of 0.028 ml/min. Treatment infusions were initiated 60 min

before the placement of a 3 mm square piece of Whatman No. 1 filter paper saturated with 35% $FeCl_3$ onto the exposed carotid artery distal to the flow probe. Treatment infusions were continued for an additional 90 minutes after the application of $FeCl_3$ (total infusion duration 150 minutes) if thrombotic occlusions did not occur, or were terminated 30 minutes after thrombotic occlusion of the vessel. Time to occlusion was defined as the time from application of $FeCl_3$ to thrombotic occlusion of the vessel. At the termination of the study (90 minutes after application of $FeCl_3$ in animals which did not occlude, or at 30 minutes after thrombotic occlusion), 3 ml blood samples were drawn by cardiac puncture into 0.3 ml of 3.8% sodium citrate.

The results show that compounds of the invention prevent thrombotic occulsions.

Thrombin Inhibitors - Therapeutic Uses

Anticoagulant therapy is indicated for the treatment and prevention of a variety of thrombotic conditions, particularly coronary artery and cerebrovascular disease. Those experienced in this field are readily aware of the circumstances requiring anticoagulant therapy. The term "patient" used herein is taken to mean mammals such as primates, including humans, sheep, horses, cattle, pigs, dogs, cats, rats, and mice.

Thrombin inhibition is useful not only in the anticoagulant therapy of individuals having thrombotic conditions, but is useful whenever inhibition of blood coagulation is required such as to prevent coagulation of stored whole blood and to prevent coagulation in other biological samples for testing or storage. Thus, the thrombin inhibitors can be added to or contacted with any medium containing or suspected of containing thrombin and in which it is desired that blood coagulation be inhibited, e.g. when contacting the mammal's blood with material selected from the group consisting of vascular grafts, stents, orthopedic prothesis, cardiac prosthesis, and extracorporeal circulation systems

The thrombin inhibitors of the invention can be administered in such oral forms as tablets, capsules (each of which includes sustained release or timed release formulations), pills, powders, granules, elixers,

tinctures, suspensions, syrups, and emulsions. Likewise, they may be administered in intravenous (bolus or infusion), intraperitoneal, subcutaneous, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts. An effective but non-toxic amount of the compound desired can be employed as an anti-aggregation agent. For treating ocular build up of fibrin, the compounds may be administered intraocularly or topically as well as orally or parenterally.

The thrombin inhibitors can be administered in the form of a depot injection or implant preparation which may be formulated in such a manner as to permit a sustained release of the active ingredient. The active ingredient can be compressed into pellets or small cylinders and implanted subcutaneously or intramuscularly as depot injections or implants. Implants may employ inert materials such as biodegradable polymers or synthetic silicones, for example, Silastic, silicone rubber or other polymers manufactured by the Dow-Corning Corporation.

The thrombin inhibitors can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines.

The thrombin inhibitors may also be delivered by the use of monoclonal antibodies as individual carriers to which the compound molecules are coupled. The thrombin inhibitors may also be combined with soluble polymers as targetable drug carriers. Such polymers can include polyvinlypyrrolidone, pyran copolymer, polyhydroxy-propyl-methacrylamide-phenol, polyhydroxyethyl-aspartamide-phenol, or polyethyleneoxide-polylysine substituted with palmitoyl residues. Furthermore, the thrombin inhibitors may be combined to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyglycolic acid, copolymers of polylactic and polyglycolic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and cross linked or amphipathic block copolymers of hydrogels.

The dosage regimen utilizing the thrombin inhibitors is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound or salt thereof employed. An ordinarily skilled physician or veterinarian can readily determine and prescribe the effective amount of the drug required to prevent, counter, or arrest the progress of the condition.

Oral dosages of the thrombin inhibitors, when used for the indicated effects, will range between about 0.1 mg per kg of body weight per day (mg/kg/day) to about 100 mg/kg/day and preferably 1.0-100 mg/kg/day and most preferably 1-20 mg/kg/day. Intravenously, the most preferred doses will range from about 0.01 to about 10 mg/kg/minute during a constant rate infusion. Advantageously, the thrombin inhibitors may be administered in divided doses of two, three, or four times daily. Furthermore, they can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, or course, be continuous rather than intermittent throughout the dosage regime.

For example, oral tablets can be prepared which contain an amount of active compound of between 100 and 500 mg, typically between 200 and 250 mg. Typically, a patient in need of thrombin inhibitor compound, depending on weight and metabolism of the patient, would be administered between about 100 and 1000 mg active compound per day. For a patient requiring 1000 mg per day, two tablets containing 250 mg of active compound can be administered in the morning and two tablets containing 250 mg of active compound can again be administered in the evening. For a patient requiring 500 mg per day, one tablet containing 250 mg of active compound can be administered in the morning and one tablet containing 250 mg of active compound can again be administered in the evening.

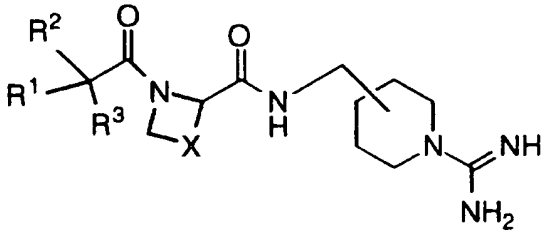
The thrombin inhibitors are typically administered as active ingredients in admixture with suitable pharmaceutical diluents, excipients or carriers (collectively referred to herein as "carrier" materials) suitably selected with respect to the intended form of administration, that is, oral tablets, capsules, elixers, syrups and the like, and consistent with convention pharmaceutical practices.

For instance, for oral administration in the form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic, pharmaceutically acceptable, inert carrier such as lactose, starch, sucrose, glucose, methyl cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, mannitol, sorbitol and the like; for oral administration in liquid form, the oral drug components can be combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, distintegrating agents and coloring agents can also be incorporated into the mixture. Suitable binders include starch, gelatin, natural sugars such as glucose or beta-lactose, corn-sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Disintegrators include, without limitation, starch methyl cellulose, agar, bentonite, xanthan gum and the like.

The thrombin inhibitors can also be co-administered with suitable anti-coagulation agents or thrombolytic agents such as plasminogen activators or streptokinase to achieve synergistic effects in the treatment of various ascular pathologies. For example, thrombin inhibitors enhance the efficiency of tissue plasminogen activator-mediated thrombolytic reperfusion. Thrombin inhibitors may be administered first following thrombus formation, and tissue plasminogen activator or other plasminogen activator is administered thereafter. They may also be combined with heparin, aspirin, or warfarin.

WHAT IS CLAIMED IS:

1. A compound having the formula:



wherein

R^1 and R^2 are independently

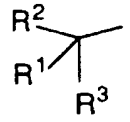
hydrogen,
phenyl unsubstituted, mono- or di-substituted with OH,
C_{1-4} alkyl, oxo, or halo,
naphthyl,
biphenyl,
a 5- to 7- membered mono- or bicyclic heterocyclic ring or bicyclic heterocyclic ring system any ring of which may be saturated or unsaturated, and which consists of carbon atoms and from one to three heteroatoms selected from the group consisting of N, O and S,
C_{1-4} alkyl,
branched C_{1-4} alkyl,
C_{3-7} cycloalkyl,
C_{5-12} bicyclic alkyl,
C_{11-16} tricyclic alkyl,
$R^4(CH_2)_n$,
$(R^4)_2CH$, wherein R^4 is the same or different,
$(R^4)(R^4O)CH$, wherein R^4 is the same or different,
$R^4O(CH_2)_n$, or
R^2 may be joined with R^1 to form a four- to seven membered carbon ring in which zero to two carbon atoms may be substituted with heteroatoms independently selected from the list N, O, and S.

where n is 1, 2, 3 or 4; and

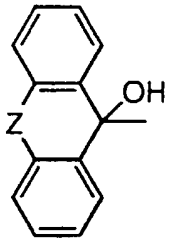
R^3 is

H,
$HO(CH_2)_p$, where p is 0, 1, 2, 3 or 4;

or



is



wherein Z is C, O or a bond;

R^4 is

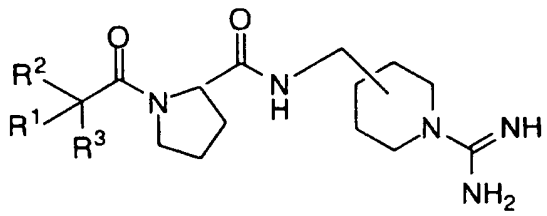
phenyl unsubstituted, mono- or di-substituted with OH, OMe, C_{1-4} alkyl, oxo, or halo,
naphthyl,
biphenyl,
1-dibenzocycloheptene,
a 5- to 7- membered mono- or bicyclic heterocyclic ring or bicyclic heterocyclic ring system any ring of which may be saturated or unsaturated, and which consists of carbon atoms and from one to three heteroatoms selected from the group consisting of N, O and S,
C_{1-4} alkyl,
branched C_{1-4} alkyl,
C_{3-7} cycloalkyl,
C_{5-12} bicyclic alkyl, or
C_{11-16} tricyclic alkyl; and

X is $(CH_2)_q$ where q is 1 or 2,
NR^1CH_2, or

SCH_2,

and pharmaceutically acceptable salts thereof.

2. A compound of Claim 1 having the structure



wherein

R^1 and R^2 are independently

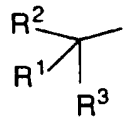
hydrogen,
phenyl unsubstituted, mono- or di-substituted with OH, C_{1-4} alkyl, oxo, or halo,
naphthyl,
biphenyl,
a 5- to 7- membered mono- or bicyclic heterocyclic ring or bicyclic heterocyclic ring system any ring of which may be saturated or unsaturated, and which consists of carbon atoms and from one to three heteroatoms selected from the group consisting of N, O and S,
C_{1-4} alkyl,
branched C_{1-4} alkyl,
C_{3-7} cycloalkyl,
C_{5-12} bicyclic alkyl,
C_{11-16} tricyclic alkyl,
$R^4(CH_2)_n$,
$(R^4)_2CH$, wherein R^4 is the same or different,
$(R^4)(R^4O)CH$, wherein R^4 is the same or different,
$R^4O(CH_2)_n$, or

R^2 may be joined with R^1 to form a four- to seven membered carbon ring in which zero to two carbon atoms may be substituted with heteroatoms independently selected from the list N, O, and S,

where n is 1, 2, 3 or 4; and
R^3 is

H,
$HO(CH_2)_p$, where p is 0, 1, 2, 3 or 4;

or



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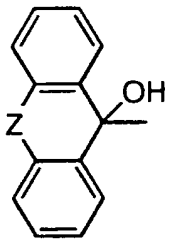


wherein Z is C, O or a bond;

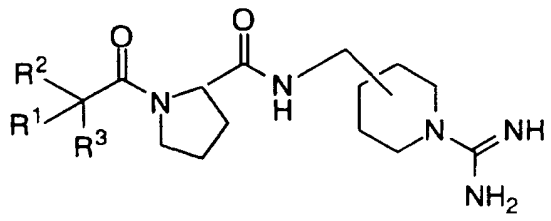
R^4 is

phenyl unsubstituted, mono- or di-substituted with OH, OMe, C_{1-4} alkyl, oxo, or halo,
naphthyl,
biphenyl,
1-dibenzocycloheptene,
a 5- to 7- membered mono- or bicyclic heterocyclic ring or bicyclic heterocyclic ring system any ring of which may be saturated or unsaturated, and which consists of carbon atoms and from one to three heteroatoms selected from the group consisting of N, O and S,
C_{1-4} alkyl,
branched C_{1-4} alkyl,
C_{3-7} cycloalkyl,
C_{5-12} bicyclic alkyl, or

C_{11-16} tricyclic alkyl,

and pharmaceutically acceptable salts thereof.

3. A compound of Claim 2 having the formula



wherein

R^1 and R^2 are independently

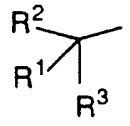
hydrogen,
phenyl unsubstituted, mono- or di-substituted with OH,
C_{1-4} alkyl, oxo, or halo,
$(R^4)_2CH$, wherein R^4 is the same or different,
R^2 may be joined with R^1 to form a four- to seven membered carbon ring in which zero to two carbon atoms may be substituted with heteroatoms independently selected from the list N, O, and S,

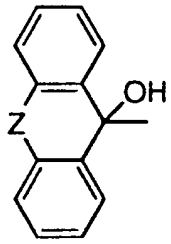
where n is 1, 2, 3 or 4; and

R^3 is

H,
$HO(CH_2)_p$, where p is 0, 1, 2, 3 or 4;

or



is 

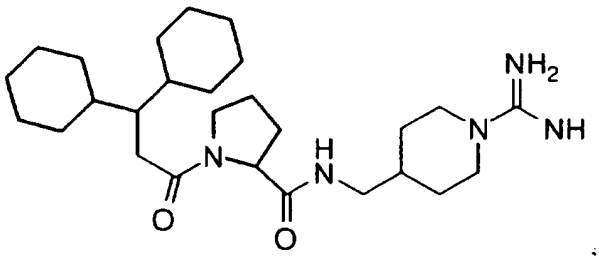
wherein Z is C, O or a bond;

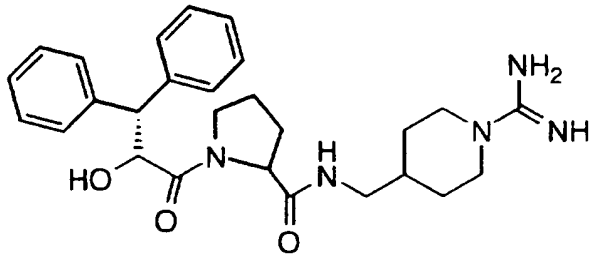
R^4 is

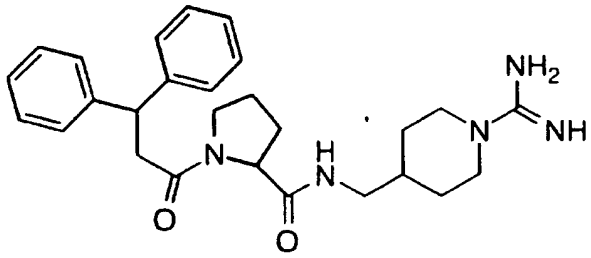
phenyl unsubstituted, mono- or di-substituted with OH, OMe, C_{1-4} alkyl, oxo, or halo,
1-dibenzocycloheptene,
a 6- membered monocyclic heterocyclic ring which may be saturated or unsaturated, and which consists of carbon atoms and from one to three N heteroatoms, or
C_{3-7} cycloalkyl,

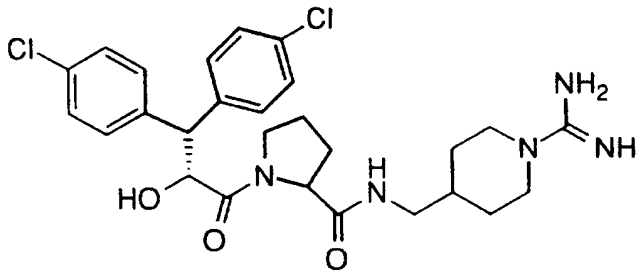
and pharmaceutically acceptable salts thereof.

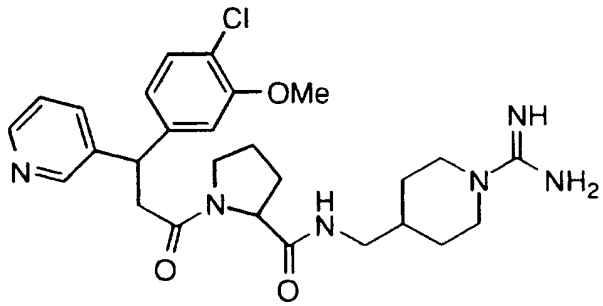
4. A compound of Claim 3 selected from the group consisting of

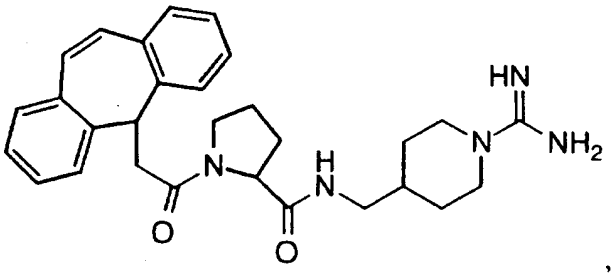


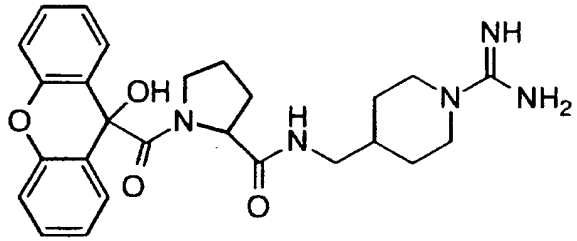


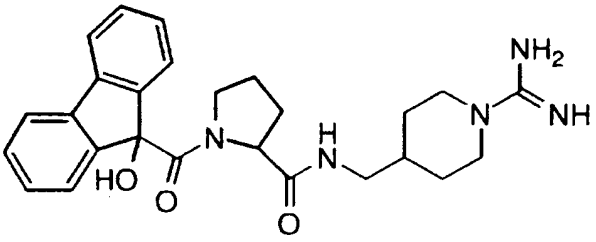







HN
NH2
N
H
N
O
O


NH
NH2
OH
O
N
H
N
O
O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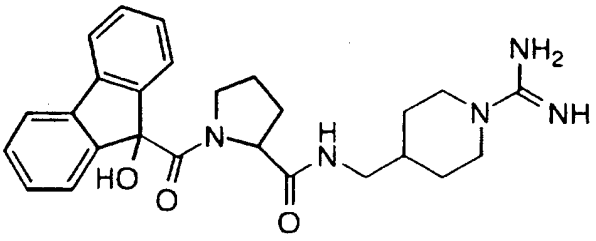
NH
HO
N
H
N
O
O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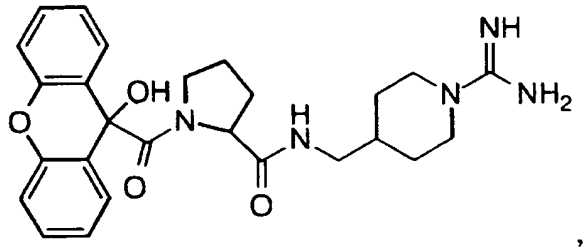
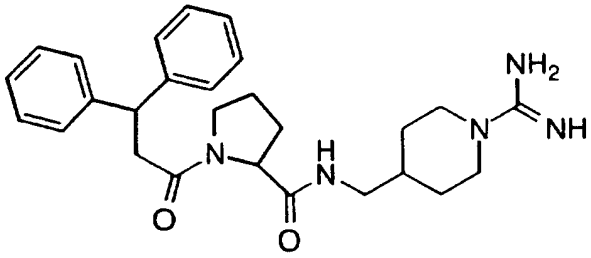
HO
N
H
N
O
O



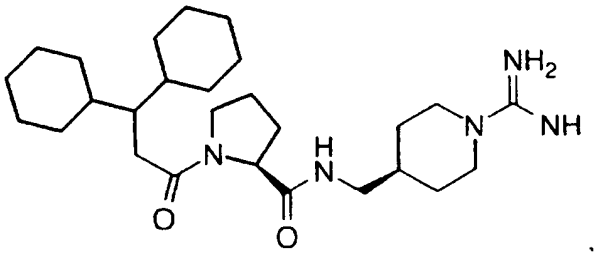
, and



.,

and pharmaceutically acceptable salts thereof.

5. A compound of Claim 4 selected from the group consisting of



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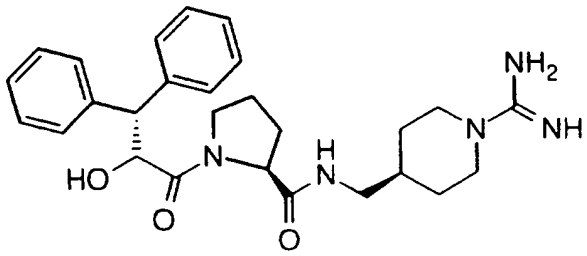

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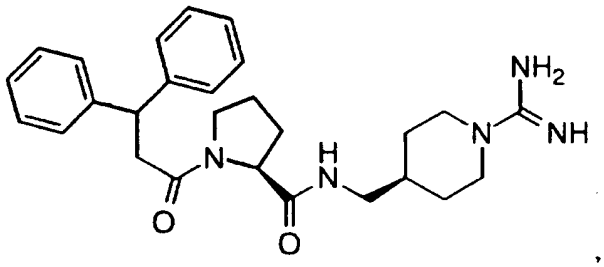

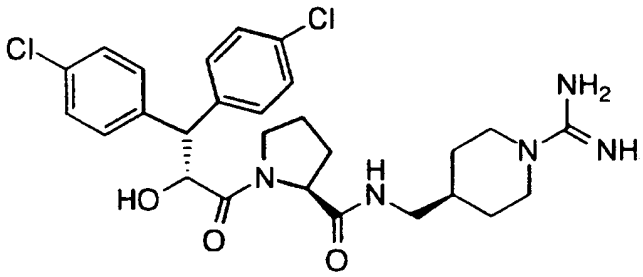

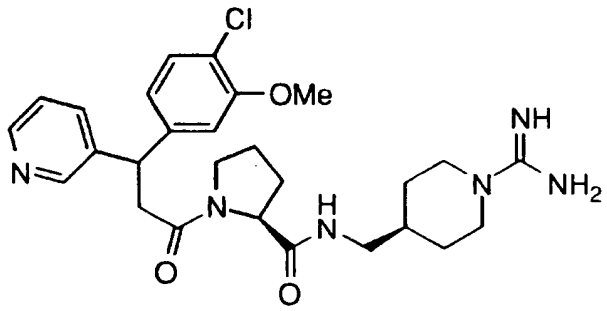

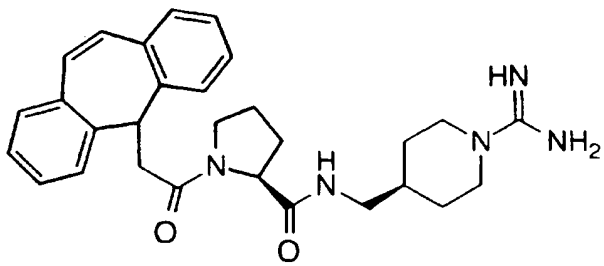

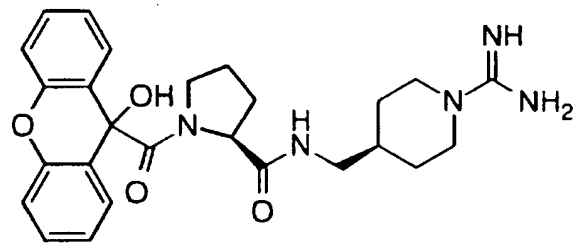

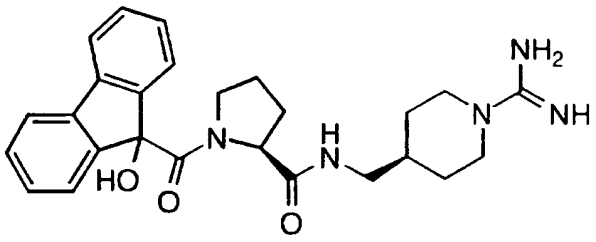

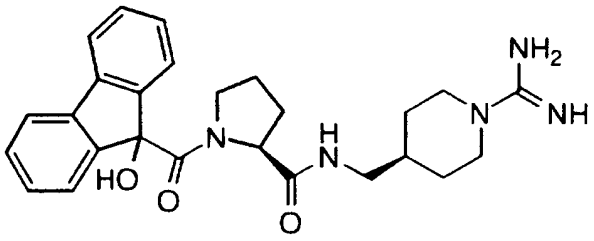

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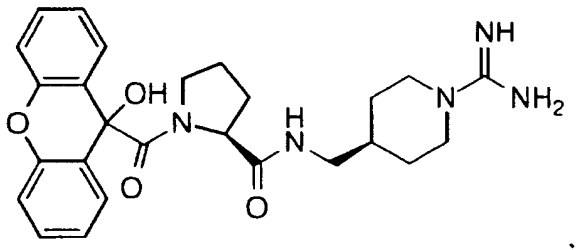

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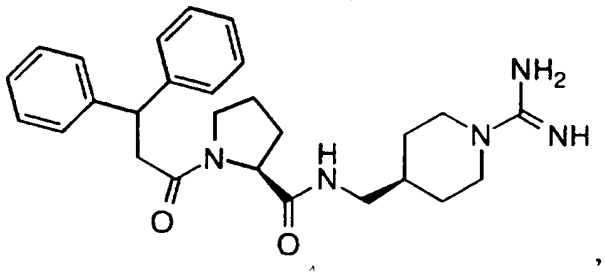
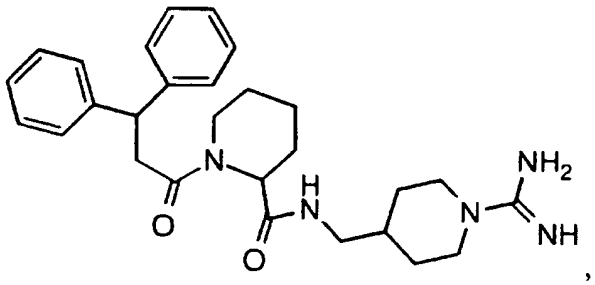


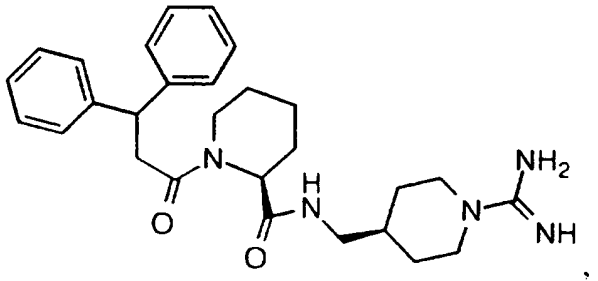
and pharmaceutically acceptable salts thereof.

6. A compound of Claim 1 which is



and pharmaceutically acceptable salts thereof.

7. A compound of Claim 6 which is



and pharmaceutically acceptable salts thereof.

8. A composition for inhibiting thrombin in blood comprising a compound of Claim 1 and a pharmaceutically acceptable carrier.

9. A composition for inhibiting formation of blood platelet aggregates in blood comprising a compound of Claim 1 and a pharmaceutically acceptable carrier.

10. A composition for inhibiting thrombus formation in blood comprising a compound of Claim 1 and a pharmaceutically acceptable carrier.

11. A method for inhibiting thrombin in blood in a mammal comprising administering to the mammal a composition of Claim 10.

12. A method for inhibiting formation of blood platelet aggregates in blood in a mammal comprising administering to the mammal a composition Claim 11.

13. A method for inhibiting thrombus formation in blood in a mammal comprising administering to the mammal a composition Claim 12.

14. The use of a compound of Claim 1, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for inhibiting thrombus formation, preventing thrombus formation, inhibiting thrombin, inhibiting formation of fibrin, and inhibiting formation of blood platelet aggregates, in a mammal.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/04679

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/445; C07D 401/06, 401/14, 405/06, 405/10

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/316, 318, 320, 325, 326; 546/187, 191, 193, 194, 196, 203, 204

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS, APS, DIALOG

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,380,713 A (BALASUBRAMANIAN ET AL.) 10 January 1995 (10.01.95), see columns 27-30, Examples 12-14.	1-14
Y, P	EP 0 648 780 A1 (BRISTOL-MYERS SQUIBB COMPANY) 19 April 1995 (19.04.95), see whole article.	1-14
A	EP 0 601 459 A2 (BRISTOL-MYERS SQUIBB COMPANY) 15 June 1994 (15.06.94), see whole article.	1-14
A	BALASUBRAMANIAN et al. Active Site-Directed Synthetic Thrombin Inhibitors: Synthesis in Vitro and in Vivo Activity Profiled of BMY 44621 and Analogs. An examination of the Role of the Amino Group in the D-Phe-Pro-Arg-H Series. J. Med. Chem. 1993, Vol. 36, No. 2, pages 300 to 303, see whole article.	1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 17 JULY 1996	Date of mailing of the international search report 09 SEP 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer CELIA CHANG Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/04679

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/04679

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/316, 318, 320, 325, 326; 546/187, 191, 193, 194, 196, 203, 204

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-10, and 14 drawn to compounds, composition and use of the compound in manufacturing a medicament.

Group II, claim 11-13, drawn to method of inhibiting thrombin, inhibiting platelet aggregation or thrombus formation.

The inventions listed as Groups I-II do not relate to a single inventive concept under PCT Rule 13.1 because unity of invention of different categories of invention will be considered to have unity for only combinations of categories:
1) A product and a process specifically adapted for the manufacture of said product; or
2) A product and process of use of said product ; or
3) A product, a process specially adapted for the manufacture of the said product, and a use of the said product; or
4) A process and an apparatus or means specifically designed for carrying out the said process; or
5) A product, a process specially adapted for the manufacture of the said product, and an apparatus or means specifically designed for carrying out the said process.

The instant application lacks unity of invention because the claims are drawn to combination (3) encompassing compounds, compositions and method of use compounds in manufacturing therapeutical formula with the additional category of method of treating specific conditions i.e. inhibiting enzyme, inhibiting platelet aggregation or thrombus formation.